

WHAT IS CLAIMED IS:

1 1. A method of testing the integrity of primers in a multiplex
2 amplification reaction, the amplification reaction comprising primers sufficient to amplify at
3 least two different target sequences, the method comprising,
4 providing in a mixture the primers and a single-stranded polynucleotide
5 sequence comprising the sequences of the primers, subsequences of the primers at least five
6 nucleotides long, or complements of the sequences of the primers;
7 amplifying the polynucleotide sequence; and
8 detecting the presence or absence of the amplified polynucleotide, thereby
9 testing the integrity of the primers in the amplification reaction.

1 2. The method of claim 1, wherein the target sequences are less than 50%
2 identical to each other.

1 3. The method of claim 1, wherein the single-stranded polynucleotide
2 sequence is provided by denaturing a double-stranded polynucleotide.

1 4. The method of claim 1, wherein the single-stranded polynucleotide
2 sequence is a synthetic single-stranded polynucleotide.

1 5. The method of claim 1, wherein the single-stranded polynucleotide
2 sequence comprises the primer sequences.

1 6. The method of claim 1, wherein the single-stranded polynucleotide
2 sequence comprises subsequences of the primers at least five nucleotides long.

1 7. The method of claim 1, wherein the single-stranded polynucleotide
2 sequence comprises all subsequences of the primers that are nine nucleotides long.

1 8. The method of claim 1, wherein the single-stranded polynucleotide
2 comprises at least two subsequences of each primer, wherein the combination of the at least
3 two subsequences contain every nucleotide of the primer sequence.

1 9. The method of claim 1, wherein the single-stranded polynucleotide
2 sequence comprises two subsequences of a primer sequence and at least the last two

3 nucleotides of a first subsequence are identical to the first at least two nucleotides of a second
4 subsequence.

1 10. The method of claim 9, wherein at least the last five nucleotides of the
2 first subsequence are identical to at least the first five nucleotides of the second subsequence.

1 11. The method of claim 1, wherein the mixture comprises at least a first,
2 second, and third primer and the single-stranded polynucleotide sequence comprises the
3 sequences of the at least first, second and third primer or subsequences at least five
4 nucleotides long of the at least first, second and third primers.

1 12. The method of claim 1, wherein the mixture comprises primers
2 sufficient to amplify at least three target sequences.

1 13. The method of claim 1, wherein the amplification of the target
2 sequences is performed in the same reaction as the amplification of the single-stranded
3 polynucleotide sequence.

1 14. The method of claim 1, wherein the mixture comprises a first primer
2 pair and the single-stranded polynucleotide sequence comprises sequences, or complement
3 thereof, of primers of the first primer pair oriented such that the first primer pair is capable of
4 amplifying the remaining primer sequences, or subsequences thereof, in the single-stranded
5 polynucleotide.

1 15. The method of claim 14, wherein the mixture comprises at least a
2 second primer pair comprising a forward and a reverse primer, wherein the single-stranded
3 polynucleotide sequence comprises sequences or subsequences of the at least second primer
4 pair oriented such that the reverse primer sequence or subsequence is closer to the 5' end of
5 the polynucleotide sequence than the forward primer sequence or subsequence.

1 16. The method of claim 15, wherein the single-stranded polynucleotide
2 sequence comprises subsequences of the primers at least five nucleotides long.

1 17. The method of claim 15, wherein the single-stranded polynucleotide
2 sequence comprises all subsequences of the primers that are nine nucleotides long.

1 18. A reagent kit, comprising

2 i. amplification reagents comprising primers sufficient to amplify at least
3 two different target sequences;

4 ii. a polynucleotide sequence comprising the sequences of the primers or
5 subsequences of the primers at least five nucleotides long; and

6 iii. at least one probe for detecting the polynucleotide sequence.

1 19. The reagent kit of claim 18, wherein the polynucleotide sequence is
2 single-stranded.

1 20. The reagent kit of claim 18, wherein the polynucleotide sequence
2 comprises the primer sequences.

1 21. The reagent kit of claim 18, wherein the polynucleotide sequence
2 comprises subsequences of the primers at least five nucleotides long.

1 22. The reagent kit of claim 18, wherein the amplification reagents
2 comprise a first primer pair and the single-stranded polynucleotide sequence comprises
3 sequences, or complement thereof, of primers of the first primer pair oriented such that
4 primer pair is capable of amplifying the remaining primer sequences, or subsequences
5 thereof, in the single-stranded polynucleotide.

1 23. The reagent kit of claim 22, wherein the amplification reagents
2 comprise at least a second primer pair comprising a forward and a reverse primer, wherein
3 the single-stranded polynucleotide sequence comprises sequences or subsequences of the at
4 least second primer pair oriented such that the reverse primer sequence or subsequence is
5 closer to the 5' end of the polynucleotide sequence than the forward primer sequence or
6 subsequence.

1 24. The reagent kit of claim 23, wherein the single-stranded polynucleotide
2 sequence comprises subsequences of the primers at least five nucleotides long.

1 25. The reagent kit of claim 18, wherein the polynucleotide sequence
2 comprises all subsequences of the primers that are nine nucleotides long.

1 26. The reagent kit of claim 18, wherein the polynucleotide sequence
2 comprises two subsequences of a primer sequence and at least the last two nucleotides of a
3 first subsequence are identical to the first at least two nucleotides of a second subsequence.

1 27. The reagent kit of claim 26, wherein at least the last five nucleotides of
2 the first subsequence are identical to at least the first five nucleotides of the second
3 subsequence.

1 28. The reagent kit of claim 18, wherein the kit comprises at least a first,
2 second, and third primer and the single-stranded polynucleotide sequence comprises the
3 sequences of the at least first, second and third primer or subsequences at least five
4 nucleotides long of the at least first, second and third primers.

1 29. The reagent kit of claim 18, wherein the reagent kit comprises a first
2 primer pair and the polynucleotide sequence comprises sequences of primers of the first
3 primer pair oriented such that the first primer pair is capable of amplifying the remaining
4 primer sequences, or subsequences thereof, in the single-stranded polynucleotide.

1 30. The reagent kit of claim 18, wherein the kit comprises at least a second
2 primer pair comprising a forward and a reverse primer, and the single-stranded
3 polynucleotide sequence comprises sequences or subsequences of the at least second primer
4 pair oriented such that the reverse primer sequence or subsequence is closer to the 5' end of
5 the polynucleotide sequence than the forward primer sequence or subsequence.

1 31. The reagent kit of claim 30, wherein the single-stranded polynucleotide
2 sequence comprises subsequences of the primers at least five nucleotides long.

1 32. The reagent kit of claim 30, wherein the single-stranded polynucleotide
2 sequence comprises all subsequences of the primers that are nine nucleotides long.